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December 4, 2019

VIA FEDERAL EXPRESS

U.S. Environmental Protection Agency
National FOIA Office
1200 Pennsylvania Ave. NW
William Jefferson Clinton Building (North)
Room 5315
Washington, DC 20004

Re: EPA FOIA REQUEST 2020-01271

Dear Ms. Veney:

Enclosed as discussed are the two documents that were omitted from our electronic EPA FOIA request. Please let me know if you require any additional information.

Sincerely,

Chris Rose

Chris Rose,
Legal Secretary to Steven E. Crick

/cir
Enclosures (2)

VOLUME 1 - TEXT

Final Report
PDSE 1192
TSIN W2004.02

TWO-YEAR REPEATED INHALATION EXPOSURE OF F344 RATS TO HM

Final Report

Submitted to:
Institute for Polyacrylate Absorbents
1330 Connecticut Ave, NW, Suite 300
Washington DC 20036-1702

Submitted by:
Inhalation Toxicology Research Institute
Lovelace Biomedical and Environmental Research Institute
P. O. Box 5890
Albuquerque, New Mexico 87185

Study Director: Janet M. Benson, PhD

ITRI Study No.: FY90-010
TSIN: W2004.02
DRD No.: PDSE 1192

Prepared for the Procter and Gamble Company under Funds-In-Agreement Number DE-FI04-86AL39741 with the Inhalation Toxicology Research Institute, which is operated for the U.S. Department of Energy under DOE Contract Number DE-AC04-76EV01013.

Final Report
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TSIN W2004.02

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EXECUTIVE SUMMARY

Equal numbers of male and female CDF (F344)/Crl BR rats were exposed to the test article, HM, by inhalation 6 hours/day, 5 days/week for up to 104 weeks to target concentrations of 0, 0.05, 0.2 or 0.8 mg HM/m^3. There were 94 male and 94 female rats assigned per HM exposure level. The rats were weighed, and detailed clinical observations were recorded on the day before HM exposures began, weekly for 13 weeks, then monthly thereafter. Groups of 10 male and 10 female rats per exposure level were designated for interim sacrifice after 26 and 52 weeks of exposure. Groups of 10 male and 10 female rats per exposure level were designated for 78 weeks of exposure followed by a 26-week "hold" period to evaluate the progression or possible resolution of HM-induced lung damage once exposures were terminated. Groups of 56 male and 56 female rats per exposure level comprised the basic study group, designated for 104 weeks of HM exposure. This latter group included six male and female surveillance rats per exposure level. Terminal sacrifices were conducted during the 105th week of the study. Basic toxicological endpoints evaluated included animal survival, clinical observations, body weight gain, clinical chemistry, hematology, urinalysis, existence of corneal and lenticular lesions, organ weight at sacrifice, and gross and histopathological changes. The effect of HM inhalation on macrophage-mediated clearance of insoluble particles (^{46}Sc-labeled polystyrene latex microspheres) from lungs was evaluated on eight males and females per exposure level after 6, 12, and 20 months of exposure.

Mean aerosol concentrations achieved during the study were within 5% of target. The aerosols were highly respirable, with the mean volume median aerodynamic diameters for all chambers ranging from 1.9 to 2.2 μm.

Animals inhaling HM for up to 104 weeks had no clinical signs of toxicity. HM inhalation produced no compound-related effects on group mean body weight, hematological or serum chemistry parameters and survival. Inhalation of 0.8 mg HM/m^3 for 26 or 52 weeks significantly increased lung weight and lung-to-body weight ratios in males and females and significantly increased tracheobronchial lymph node (TBLN) and TBLN-to-body weight ratios in females. Female rats inhaling 0.8 mg HM/m^3 for 104 weeks had significantly increased lung weights and lung-to-body weight ratios.

Toxic effects resulting from HM inhalation were restricted to the lung and the tracheobronchial lymph nodes. Chronic exposure of rats to 0.2 and 0.8 mg HM/m^3 resulted in inflammatory and proliferative lesions. Exposure to 0.8 mg HM/m^3 also resulted in significant increases in neoplastic lesions. HM-induced inflammatory and proliferative lesions were present in both interim and terminal sacrifice rats and persisted in some rats 26 weeks after the end of 78 weeks of HM exposure. The principal nonneoplastic lesions, found only in rats exposed to 0.2 and 0.8 mg HM/m^3, consisted of centriacinar alveolitis, alveolar macrophage hyperplasia, and alveolar epithelial hyperplasia. The severities of the inflammatory and proliferative lesions were often greater in females than males.

Compound-related neoplastic lesions were evident in the lungs of both male and female rats of the basic study group exposed to 0.8 mg HM/m^3. These neoplastic lesions consisted of bronchiolar/alveolar adenomas and bronchiolar/alveolar adenocarcinomas. The incidence of bronchiolar/alveolar adenocarcinomas was significantly increased in females. The combined incidences of bronchiolar/alveolar adenomas and bronchiolar/alveolar adenocarcinomas were significantly increased in both males and females, but the incidences were greater among female.

Alveolar macrophage-mediated clearance of inhaled particles from lungs of HM-exposed rats was evaluated after 6, 12, and 20 months of exposure. Modest, but statistically significant impairment of particle clearance occurred only in females inhaling the ^{46}Sc-labeled test particles after 20 months of exposure to 0.8 mg HM/m^3.

In summary, inhalation of 0.05, 0.2, or 0.8 mg HM/m^3 6 hours/day, 5 days/week by male and female rats for up to 104 weeks resulted in no biologically relevant compound-related effects on body weight, hematological parameters, serum chemistry, or survival and produced no signs of clinical toxicity. Alveolar macrophage-mediated particle clearance was impaired only in female rats exposed to the test particles after inhaling 0.8 mg HM/m^3 for 20 months. Lesions attributable to HM exposure were confined to the lungs and the tracheobronchial lymph node. Inflammatory and proliferative nonneoplastic lesions were seen in rats exposed to 0.2 and 0.8 mg HM/m^3, while compound-related neoplastic lung lesions occurred only in males and females exposed to 0.8 mg HM/m^3. Because there were no compound-related lesions observed in male or female rats of the 0.05 mg HM/m^3 group, this concentration of HM is interpreted as a no-observed effect level.

IPA

INSTITUTE FOR POLYACRYLATE ABSORBENTS, INC.

1100 New York Ave., N.W. • Suite 1090 • Washington, D.C. 20005 (202) 414-4190 • fax (202) 289-8584

February 7, 1997

Dr. Vanessa Vu
Office of Pollution Prevention and Toxics
US Environmental Protection Agency
401 M Street, SW (TS 794)
Washington, DC 20460

Dear Dr. Vu:

The attached report updates the final report on the polyacrylate ("HM") polymer chronic inhalation study conducted by Lovelace Inhalation Toxicology Research Institute sponsored by the Institute for Polyacrylate Absorbents, Inc. (IPA).

When the results of the HM chronic inhalation study were reported to the EPA, IPA noted its plans to conduct additional analyses to gain a more thorough understanding of the study results. IPA initiated further evaluations of the brain tissues from the basic study group rats (exposed to 0.05 and 0.2 mg HM/m^3) and additional statistical analyses of the particle clearance data on lung clearance.

The results of these additional analyses are contained in the attached final report addendum. The additional work confirmed the conclusions of the original chronic inhalation study report:

- Occurrence of astrocytomas noted in the original evaluation was not related to HM exposure.
- PA exposure had no effect on the long-term particle clearance in either males or females.

IPA hopes this additional information is helpful. If you would like to discuss this report in further detail, please contact Dr. Mark Lafranconi (513-627-4435).

Sincerely,

Richard E. Opatick

Richard E. Opatick
Executive Director

attach.

ADDENDUM TO:
TWO-YEAR REPEATED INHALATION EXPOSURE OF F344 RATS TO HM

Final Report Addendum

Submitted to:

Institute for Polyacrylate Absorbents

1100 New York Ave., Suite 1090

Washington DC 20005

Submitted by:

Inhalation Toxicology Research Institute

Lovelace Biomedical and Environmental Research Institute

P. O. Box 5890

Albuquerque, New Mexico 87185

Study Director: Janet M. Benson, PhD

ITRI Study No.: FY90-010

TSIN: W2004.02

DRD No.: PDSE 1192

Prepared for the Institute for Polyacrylate Absorbents under Funds-In-Agreement Number DE-FI04-95AL87328 with the Inhalation Toxicology Research Institute, which is operated for the U.S. Department of Energy under DOE Contract Number DE-AC04-76EV01013.

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COMPLIANCE STATEMENT

This study addendum was conducted in compliance with the Environmental Protection Agency Good Laboratory Practice Standards as set forth in Title 40 of the U.S. Code of Federal Regulations Part 792 issued November 29, 1983 (revised August 17, 1989). There were no deviations from the aforementioned standards which affected the quality of integrity of the study or the interpretation of the results in the report.

ARCHIVAL OF STUDY RECORDS

All ITRI records and specimens resulting from this study addendum will be retained at ITRI until written acceptance by the Sponsor. These materials under seal will be transferred to the archives of The Procter & Gamble Company, the study co-sponsor, for retention in compliance with 40 CFR 792.195. Should inspection of the study be necessary, the sealed records will be sent back to the ITRI within 5 days of receipt of the request.

Protocol Amendment Signed by Study Director: 10-5-94

Protocol Amendment Signed by Sponsor Representative: 12-14-94

Janet M. Benson 10/14/96

Janet M. Benson, PhD, DABT
Study Director, ITRI

Date

CONTRIBUTING PERSONNEL

Janet M. Benson, PhD, DABT	Study Director (8/10/90 to present)
Charles H. Hobbs, DVM, DABT	ITRI Directorate Member Responsible for Study
Jack R. Harkema, DVM, PhD, ACVP	Pathologist
M. Burton Snipes, PhD, DABT	Toxicokineticist
William C. Griffith, PhD	Statistician
Dorothy L. Harris, MS	Quality Assurance Officer

QUALITY ASSURANCE STATEMENT

Addendum to: Two-Year Repeated Inhalation Exposure of F344 Rats to HM

This study addendum report was inspected by the Inhalation Toxicology Research Institute Quality Assurance Unit. Findings were discussed and written comments submitted to the Study Director and to management.

QA Unit Schedule of Inspections

Study Phase	Inspection Date	Report Date
Protocol Amendment	11-15-94	11-15-94
Draft Report Addendum Audit	8/10/95	8/10/95

Dorothy Harris

Dorothy L. Harris, MS
Quality Assurance Officer

10/15/96
Date

APPROVAL SHEET

Addendum to: Two-Year Repeated Inhalation Exposure of F344 Rats to HM

Janet M. Benson 10/14/96

Janet M. Benson, PhD, DABT — Date
Study Director

Charles H. Hobbs 10/14/96

Charles H. Hobbs, DVM, DABT — Date
Associate Director with
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Morris B. Snipes 10/14/96

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William C. Griffith, PhD — Date
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Final Report Addendum
PDSE 1192
TSIN W2004.02

EXECUTIVE SUMMARY

The Inhalation Toxicology Research Institute recently completed a two-year inhalation study evaluating the effects associated with repeated inhalation of HM, a hygroscopic polymer widely used in industry ("Two-Year Inhalation Exposure of Rats to HM, Final Report, June, 1994). Among the findings of the study was a slight increase in incidence of astrocytomas among basic study group male rats exposed to 0.8 mg HM/m^3 compared to controls and an oligodendroglioma in one control male rat in the basic study group. Based on these rare findings, the Institute for Polyacrylate Absorbents (IPA) requested complete evaluations of brain tissue from basic study group rats exposed to 0.05 and 0.2 mg HM/m^3.

As a result of the additional histopathological evaluations, three new primary tumors were found in male rats and no additional neoplastic lesions were found among the females. One of 24 male rats exposed to 0.05 mg HM/m^3 evaluated in the additional work had a solitary glioma while the other male rat from this exposure group had a granular cell tumor. One of 20 males exposed to 0.2 mg HM/m^3 evaluated in the additional work had a solitary astrocytoma, bringing the overall total for the study to four. A few nonneoplastic lesions in males and females consisting chiefly of compression secondary to the presence of pituitary tumors were not considered to be related to HM inhalation.

Statistical evaluation of the revised individual and combined glial tumor (astrocytoma, glioma, oligodendroglioma) data from the original report and this additional work combined indicated that the incidence of such tumors was not significantly greater than control values in male or female rats. The incidence of astrocytomas alone and in combination with glial tumors among males was not considered to be statistically increased above background. It

was concluded that the results of the additional brain tissues evaluations do not alter the conclusions of the original report that the occurrence of the astrocytomas was not related to HM exposure.

The effect of HM inhalation on macrophage-mediated clearance of surrogate insoluble latex particles was also evaluated in subgroups of rats after 6, 12, and 20 months of inhaling 0, 0.05, 0.2, or 0.8 mg HM/m^3. In the original report a modest, but statistically significant impairment of particle clearance occurred only in females inhaling the test particles after 20 months of exposure to 0.8 mg HM/m^3. Based on these findings, the IPA also requested that the ITRI perform additional statistical evaluations of the particle clearance data, focusing on clearance occurring beyond 40 days after inhalation of the tracer particles.

Further evaluation of the clearance data showed the rate of long term particle clearance depended only on gender, being slower in females, but was not affected by HM inhalation. However, the percentage of the initial lung burden available for long-term clearance was greater in ageing females than males and the percentage increased in both males and females as a function of HM dose (HM/m^3-months). The magnitude of the HM dose effect was approximately the same in males and females. These results suggest that HM inhalation reduced the rate of short-term particle clearance in male and female rats.

I. ADDITIONAL HISTOPATHOLOGICAL EVALUATION OF BRAIN LESIONS

A. Introduction

The Inhalation Toxicology Research Institute recently completed a two-year inhalation study evaluating the effects associated with repeated inhalation of HM, a hygroscopic polymer widely used in industry ("Two-Year Repeated Inhalation Exposure of Rats to HM, Final Report," June 1994). The toxic effects associated with repeated HM inhalation were confined to the lung. However, there was an increased incidence of astrocytomas among basic study group male rats exposed to 0.8 mg HM/m^3 compared to controls. There was also an oligodendroglioma present in one control male rat in the basic study group. In order to obtain complete information on the incidence of primary brain tumors among basic study group rats, the Sponsor requested that we conduct evaluations of the brain tissue from the remaining basic study group rats exposed to 0.05 and 0.2 mg HM/m^3. These evaluations and the results obtained are described below. The amendment to the study protocol is given in Appendix A.

B. Methods

Microscopic evaluations of brain sections were performed on 44 male and 61 female basic study group rats that had been exposed to 0.05 and 0.2 mg HM/m^3. Three standard transverse levels of brain were examined in each animal following procedures previously described ("Two-Year Repeated Inhalation Exposure of F344 Rats to HM, Final Report," June 1994). Level I contained the cerebral cortex, lateral ventricles, corpus callosum, and caudate putamen. Level II contained the cerebral cortex, thalamus, third ventricle, and hippocampus. The cerebellum, medulla, fourth ventricle, and pyramidal tracts

were present in Level III of the brain. The brain sections were examined for both neoplastic and nonneoplastic lesions.

Pituitary tumors were considered endocrine tumors. The presence of neoplastic mononuclear cells characteristic of mononuclear cell leukemia was not considered indicative of a primary brain tumor if mononuclear cell leukemia was also present in other organs of the animal. Granular cell tumors of the brain, although primary brain tumors, were considered to arise from the meninges rather than being primary glial tumors. Thus, granular cell tumors were not combined with glial tumors of the brain for analysis.

Statistical evaluations of the incidences of neoplastic lesions were performed as described in the original study report. The analyses were conducted on data generated in the original study in combination with the data from this additional work.

C. Results

1. Nonneoplastic Brain Lesions

The male and female rats evaluated under this protocol amendment are listed in Appendix B. Nonneoplastic brain lesions in all basic study group males and females are in Table I-1. This table is a combination of findings from the original report as well as the findings from the additional work. No additional nonneoplastic lesions were present in brains of male rats exposed to 0.05 mg HM/m^3. The findings in this group are the same as presented in the original report. Brain lesions were present in 2/20 male rats exposed to 0.2 mg HM/m^3 evaluated in this additional work. Minimal, focal inflammation in the choroid plexus was evident in one rat (C189). Moderate compression of the hypothalamus with secondary hydrocephalus caused by a large pituitary adenoma was found in another rat

(C271). Brain lesions were found in 1/34 females exposed to 0.05 mg HM/m^3 and 2/27 females exposed to 0.2 mg HM/m^3 evaluated in this additional work. One female (B549) exposed to 0.05 mg HM/m^3 had minimal compression of the hypothalamus secondary to a pituitary tumor. Pituitary tumor-induced compression of the hypothalamus of minimal severity was also evident in the two female rats (C647, C649) exposed to 0.2 mg HM/m^3. All three animals (B549, C647, C649) had minimal compression of the hypothalamus secondary to the presence of a pituitary tumor.

2. Neoplastic Brain Lesions

The data in Table I-2 are combined values from the original study report and the additional work conducted under Protocol Amendment 9. The results below are directed to the findings from the additional work. Additional primary brain tumors were found in 2/24 male rats exposed to 0.05 mg HM/m^3 and 1/20 male rats exposed to 0.2 mg HM/m^3. One rat in the 0.05 mg/m^3 group (B177) had a solitary glioma in the medulla located in level III of the brain. The other rat (B133) had a solitary granular cell tumor associated with the meninges of the dorsal median sulcus in level I of the brain. One rat (C198) exposed to 0.2 mg/m^3 had a solitary astrocytoma that was restricted to the ventral aspect of the medulla in level III of the brain. No female rats and no other male rats evaluated under the amendment had neoplastic brain lesions.

One rat male rat (B101) exposed to 0.05 mg HM/m^3 contained mononuclear cells that were restricted to the blood vessels throughout the brain. Neoplastic mononuclear cells characteristic of mononuclear cell leukemia were found in several other

organs in this animal. An infiltration of neoplastic cells into the brain tissue, however was not evident in the sections examined.

D. Discussion

In the original two-year HM inhalation study in rats, astrocytomas of the brain were observed in 3/56 (5.4%) of basic study group male rats exposed to 0.8 mg HM/m^3. Astrocytomas were not observed in basic study group control male and female rats, in basic study group females exposed to 0.8 mg HM/m^3, or in basic study group males and females exposed to 0.05 or 0.2 mg HM/m^3 which were sacrificed when moribund. A single oligodendroglioma was also present in a control male in the basic study group. Because the incidence of astrocytomas in the 0.8 mg HM/m^3 males was not considered to be statistically increased above background and because there was no evidence of potentially preneoplastic lesions usually seen with induced brain tumors in any of the HM-exposed groups, the occurrence of astrocytomas was not considered to be related to HM exposure. Nonneoplastic brain lesions observed included compression, inflammation, hydrocephalus, and necrosis. There was no relationship between the incidence of these lesions and HM exposure concentration.

One of the chief purposes for this addendum to the two-year HM study in rats was to obtain complete information on the incidence of brain lesions, especially glial tumors, in previously unevaluated rats exposed to 0.05 and 0.2 mg HM/m^3. As a result of these additional evaluations, three new primary brain tumors were found in male rats. No neoplastic lesions were found among the females examined. One of 24 male rats exposed to 0.05 mg HM/m^3 had a solitary glioma in level II of the brain, while the other male rat from

this exposure group had a granular cell tumor in level I. One of 20 males exposed to 0.2 mg HM/m^3 had a solitary astrocytoma in level III of the brain, bringing the overall total for the study to 4. A few additional nonneoplastic lesions were observed among males and females. These consisted chiefly of compression secondary to the presence of pituitary tumors and were not considered to be related to HM exposure.

The incidences of primary brain tumors in the additional rats were low (similar to the incidences for spontaneous brain tumors in historical controls for F344 rats) and the histologic characteristics of the individual brain tumors were not different from those described for spontaneous brain tumors in F344 rats (Boorman, 1980).

The incidence of astrocytomas among all basic study animals was not consistently statistically significant. Importantly, as was the case in the original statistical analysis of the astrocytoma data, the incidences in the 0.2 and 0.8 mg HM/m^3 exposure groups was not significant by the life table test. Statistical evaluation of the revised individual and combined glial tumors (astrocytoma, glioma, oligodendroglioma) incidence data (to include all basic study animals on which histopathology was performed) also indicated the incidences of such tumors were not significantly greater than control values in either male or female rats. Since the overall incidence of astrocytomas alone and in combination with other glial tumors among males was not considered to be statistically increased above background, and because there was no evidence of potentially preneoplastic lesions usually seen with induced brain tumors in any of the HM-exposed groups, the occurrence of glial neoplasms of any type was not considered to be related to HM exposure.

Therefore, the results of the additional brain tissue evaluations do not alter the conclusions of the original report that the occurrence of the astrocytomas was not related to HM exposure.

II. ADDITIONAL EVALUATION OF TRACER PARTICLE CLEARANCE

A. Introduction

The Inhalation Toxicology Research Institute recently completed a two-year inhalation study evaluating the effects associated with repeated inhalation of HM, a hygroscopic polymer widely used in industry ("Two-Year Repeated Inhalation Exposure to HM, Final Report, June 1994). One of the parameters evaluated during this study was the effect of HM inhalation on macrophage mediated particle clearance. The pulmonary clearance of acutely inhaled ^{46}Sc-labeled polystyrene latex (PSL) microspheres of about 4 μm geometric diameter, was determined on the same subgroups of rats exposed to 0, 0.05, 0.2, and 0.8 mg HM/m^3 after 6, 12, and 20 months of chronic HM inhalation. Two-component negative exponential functions were fit to the data. The data from the HM control and exposed groups were compared using multivariate analysis of variance and all interpretations regarding impairment or lack of impairment of ^{46}Sc-PSL particle clearance were made based on the results of these statistical analyses. Modest, but statistically significant impairment of particle clearance occurred only in females inhaling test particles after 20 months of exposure to 0.8 mg HM/m^3 (the highest HM aerosol concentration tested).

Mathematical modeling of lung particle clearance data typically resolves the deep lung or alveolar clearance of poorly soluble particles in rat lungs into early and late components. The early component typically exhibiting a half-time of 20-30 days and the later

component typically with a half-time of greater than 100 days. The primary mechanism of clearance of poorly soluble particles in both early and late periods is believed to be macrophage transport of particles from the alveolar and interstitial regions of the lung to the airways or to lung-associated lymph nodes and particle dissolution. In the HM chronic inhalation study alveolar clearance was characterized by exposure of rats to radioactively tagged latex particles. The initial analysis of the tracer particle clearance data indicated an impairment of deep lung clearance in female rats exposed to 0.8 mg HM/m^3 for 20 months. To better understand the effect of chronic HM inhalation on particle clearance in the rat lung, the Institute for Polyacrylate Absorbents (IPA) requested that ITRI perform additional statistical evaluations on the tracer particle clearance data. The additional analyses focused on the effects of HM inhalation on the later period of alveolar clearance defined in this report as that occurring in the time period beyond 40 days after tracer particle exposure.

Specifically, the IPA requested statistical comparisons of these data as a function of HM aerosol concentration for each time the insoluble particles were administered (6, 12, and 20 months of HM inhalation) and across all time points. These evaluations and the results obtained are described below.

B. Methods

Particle burdens from 40–120 days after exposure to the tracer particles were used to evaluate long-term clearance. A random effects model for longitudinal data (Laird and Ware, 1982) was used to analyze the particle clearance data. This method was used because the experiment involved multiple measurements of pulmonary retention in individual animals exposed to tracer particles at three different times after the HM exposures began.

Long-term retention functions for each evaluation of clearance at 6, 12, and 20 months which will be referred to as age, were estimated using linear regression on log transformed data. The intercept of the long-term retention function represents the fraction of the initial lung burden of tracer particles remaining in the lung after short-term clearance was complete. This fraction was available for long-term clearance. The slope of the function represents the long-term clearance rate. Initially the median and range of intercepts and slopes associated with long term clearance were evaluated as a function of exposure concentration, animal age and gender. This involved fitting each set of retention data separately, then the effect of the population covariates of animal age, gender, and HM dose (concentration of HM x exposure duration) on the fraction of material available for long-term clearance (intercept) and the long-term clearance rate (slope) were evaluated using a random effects analysis method.

In this type of random effects analysis (where there are multiple measurements at different times on individual animal) the results can be viewed as consisting of two parts. The first part is the rate of long-term clearance and the fraction available for long term clearance for each animal, at each age. The second part of the random effects analysis is the dependence of the above parameters on gender, dose, age, and interactions between these factors. These two parts of the analysis estimate "within animal variability" and "between animal variability". The "within animal variability" is the variability of the individual retention measurements for an animal about its retention function. The "between animal variability" is the variability of the rates of clearance and the fraction of particles available among the animals after any statistically significant systematic effects of gender, dose, age and their interactions are taken into account. The statistical significance of systematic effects

of gender, dose, age and their interactions is determined using the Akiake (reference) information criteria.

C. Results

The median and range of rates of long-term clearance and fraction of particles available for long-term clearance are shown in Table II-1 for each retention data set. Males and females appear to have a different pattern of change in the long-term clearance with increasing age and increasing dose. For males, there appears to be little significant change with age (comparing data across rows), and only a small difference with increasing dose (comparing data down columns). For females there is a longer retention time, larger increases in the long-term clearance with increasing age and increasing dose.

Because the rate of clearance and percentage of initial lung burden available for clearance are highly correlated with each other for long term retention, it is difficult to interpret the changes in Table II-1 except in a model where the rates of long-term clearance and percentage of particles available for clearance are linked together. To sort out the effects of age, gender, dose, and their interactions it is necessary to apply the random effects model, where, for purposes of describing the effects of these parameters, age and dose are assumed to have a linear trend.

The random effects model was applied to the retention data to determine the effect of gender, dose, and age on the rates of long term clearance and fraction of particles available. The random effects model showed that the rate of long term clearance only depended on gender. The percentage of initial tracer particle burden available for long term clearance depended on gender, dose, and age and the interaction between gender and age.

The estimated rate of long-term clearance for the females had a clearance half-time of 122 days with a 95% confidence interval of 100–155 days, and for the males a clearance half-time of 49 days with a 95% confidence interval of 45–54 days. HM inhalation did not affect the rate of long-term clearance of either sex. The standard error of the individual retention measurements about the individual retention functions (within animal variability) was about 19%.

The model also indicated that the percentage of the initial lung burden available for long term clearance was affected by age in females and by HM dose in both males and females (Table II-2). The magnitude of the dose-effect was approximately the same in males and females. For example, the ratios of the percentage of the initial particle burden available for long-term clearance in males and females with a dose of 16 mg/m^3-month compared to control males and females were 1.6 and 1.7, respectively.

D. Discussion

The estimated half-times for long-term pulmonary particle clearance in control males and females in this study determined using random effects analysis were 49 and 122 days, respectively. The results of this analyses indicate the rate of long-term pulmonary particle clearance is significantly slower for females than males. It is difficult to directly compare the rates of long-term clearance from this study with those of other published studies because the time over which long term clearance is defined (greater than 40 days post exposure) is unique.

In contrast to the results of the original statistical analyses of the pulmonary tracer particle clearance data indicating that inhalation of 0.8 mg HM/m^3 for over 20 months

impaired tracer particle clearance in female rats, the additional statistical evaluations focusing on long-term clearance indicate that HM inhalation had no effect on the rate of long-term particle clearance in either males or females. However, the additional evaluations indicated that the percentage of the initial particle burden available for long-term clearance is increased in ageing females, but not males. The results further indicate that the percentage of initial particle burden available for the later period of alveolar clearance is increased with HM dose in both males and females. The increased percentage of tracer particles associated with late alveolar clearance is likely the result of an HM-induced decrease in early alveolar clearance and/or increased distribution of tracer particles into slower clearing compartments such as the interstitium and lymph nodes. Increased interstitialization and lymph node accumulation of poorly soluble particles has been reported to occur when lung particle burdens exceed 1 mg (Ferin and Feldstein, 1978; Snipes, 1989), which is consistent with the projected lung burdens of hydrated HM at the high exposure level in the chronic study.

III. REFERENCES

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Snipes, M. B. (1989). Long-term retention and clearance of particles inhaled by mammalian species. *Crit. Rev. Toxicol. 20*: 175.

Table I-1

Incidences of Selected Nonneoplastic Brain Lesions in the Basic Study Group
Two-Year Repeated Inhalation Exposure of F344 Rats to HM[a]

	Males				Females			
	0[b]	0.05	0.2	0.8[b]	0[b]	0.05	0.2	0.8[b]
Number of Brains Examined	56	56	56	56	56	56	56	56
Compression	5[c] (3.4)[d]	1 (4)	4 (3.5)	2 (3)	3 (2.7)	5 (2.8)	6 (2.3)	3 (3)
Hemorrhage	7 (PNG)[c]	3 (PNG)	2 (PNG)	2 (PNG)	3 (3.3)	1 (2)	1 (4)	0
Necrosis	3 (4)	1 (3)	0	0	1 (4)	0	0	0
Inflammation	0	0	1 (1)	0	1 (4)	0	0	0
Hydrocephalus	0	0	1 (3)	0	1 (2)	2 (3)	1 (3)	2 (3)

[a]The data in this table are combined data from those generated in the original report and those from the original work.
[b]These data were provided in the original report and are included here for reference.
[c]Number of animals with lesions
[d]Average severity grade of lesions in affected animals; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, PNG = present but not graded.

Table I-2

Incidences of Brain Neoplasms in the Basic Study Group
Two-Year Repeated Inhalation Exposure of F344 Rats to HM[a]

	Males			
	0 mg[b] HM/m^3	0.05 mg HM/m^3	0.2 mg HM/m^3	0.8 mg[b] HM/m^3
Basic Study Group (No. of Animals)	56	56	56	56
Oligodendroglioma				
Overall rates[c]	1/54 (2%)	0/56	0/56	0/56
Terminal rates[d]	0/27	0/24	0/21	0/28
First incidence (days)	546 (M)	NA	NA	NA
Logistic regression test[e]	0.456			
Hoel-Walburg prevalence test[f]		0.157	0.317	0.617
Fisher's exact test[f]		0.491	0.491	0.491
Life table test		0.516	0.508	0.485
Astrocytoma				
Overall rates[c]	0/54	0/56	1/56 (2%)	3/56 (5%)
Terminal rates[d]	0/27	0/24	1/21 (5%)	0/28
First incidence (days)	NA	NA	730 (T)	437 (M)
Logistic regression test[e]	0.234			
Hoel-Walburg prevalence test[f]			0.275	0.028
Fisher's exact test[f]			1.00	0.243
Life table test			0.467	0.145
Granular Cell Tumor				
Overall rates[c]	0/54	1/56 (2%)	0/56	0/56
Terminal rates[d]	0/27	1/24 (4%)	0/21	0/28
First incidence (days)	NA	729 (T)	NA	NA
Logistic regression test[e]	0.484			
Hoel-Walburg prevalence test[f]		0.307		
Fisher's exact test[f]		1.00		
Life table test				

Table I-2 (Concluded)

Incidences of Brain Neoplasms in the Basic Study Group
Two-Year Repeated Inhalation Exposure of F344 Rats to HM[a]

	Males			
	0 mg[b] HM/m^3	0.05 mg HM/m^3	0.2 mg HM/m^3	0.8 mg[b] HM/m^3
Glioma				
Overall rates[c]	0/54	1/56 (2%)	0/56	0/56
Terminal rates[d]	0/27	1/24 (4%)	0/21	0/28
First incidence (days)	NA	730 (T)	NA	NA
Logistic regression test[e]	0.485			
Hoel-Walburg prevalence test[f]		0.307		
Fisher's exact test[f]		1.00		
Life table test		0.484		
All Glial Tumors Combined				
Overall rates[c]	1/54 (2%)	1/56 (2%)	1/56 (2%)	3/56 (5%)
Terminal rates[d]	0/54	1/24 (4%)	1/21 (5%)	0/28
First incidence (days)	546 (M)	729 (T)	730 (T)	437 (M)
Logistic regression test[e]	0.211			
Hoel-Walburg prevalence test[f]		0.820	0.951	0.067
Fisher's exact test[f]		1.00	1.00	0.618

[a]The data in this table are combined data from those generated in the original report and those from the original work. (M) Moribund Sacrifice - (T) Terminal Sacrifice
[b]Data for these rats were presented in the original report and are included here for reference.
[c]Number of tumor-bearing animals/number of animals examined microscopically
[d]Observed incidence at terminal kill
[e]Logistic regression test is a trend test to determine if there is a statistically significant linear trend on a logistic scale across all exposure concentrations.
[f]Beneath the dosed group incidence are the
p values corresponding to pairwise comparisons between the controls and that dosed group by the Hoel-Walburg and by the Fisher's exact test.

Table II-1

Median and Ranges for Lung Term Clearance Parameters for Each Retention Data Set Estimated Separately. These are Categorized by Gender, HM Dose, and Age.

Exposure Concentration (mg HM/m^3)	Age							
	6 months			12 months			20 months	
Dose mg/m3 month	Percentage[a]	Half time (days)	Dose mg/m3 month	Percentage	Half time (days)	Dose mg/m3 month	Percentage	Half time (days)
Males								
0	14 (7,49)[b]	49 (27, 167)	0	13 (6, 23)	56 (26, 102)	0	11 (9, 14)	70 (48, 108)
0.3	13 (9, 25)	50 (26, 271)	0.6	17 (12, 35)	51 (25, 95)	1.0	13 (3, 28)	51 (31, 65)
1.2	15 (6, 30)	48 (35, 110)	2.4	18 (9, 36)	40 (27, 93)	4.0	7 (5, 27)	45 (25, 4744)
4.8	13 (6, 31)	50 (38, 70)	9.6	15 (11, 28)	63 (52, 126)	16.0	24 (9, 42)	67 (31, 133)
Females								
0	14 (4, 28)	80 (49, 3880)	0	25 (13, 34)	96 (64, 245)	0	33 (15, 44)	94 (63, 100)
0.3	17 (8, 33)	162 (84, 224)	0.6	30 (21, 42)	82 (51, 133)	1.0	39 (35, 52)	85 (73, 103)
1.2	21 (7, 51)	186 (98, 260)	2.4	25 (16, 47)	152 (90, 268)	4.0	42 (28, 65)	122 (72, 193)
4.8	11 (5, 53)	312 (-66, 4857)	9.6	26 (23, 55)	112 (52, 1876)	16.0	43 (33, 53)	419 (241, 765)

[a]Percentage of initial particle burden available for long term clearance.
[b]Values are the median (range) of intercept and clearance half-time.

Table II-2

Estimated Percentage of Initial Particle Burden Available
for Long-Term Clearance and 95% Confidence Intervals in Random Effects Model

Exposure Concentration (mg HM/m^3)	Age					
	6 Months		12 Months		20 Months	
	Dose (mg/m^3-months)	Percentage[a]	Dose (mg/m^3-months)	Percentage[a]	Dose (mg/m^3-month)	Percentage[a]
Males						
0	0	15.2 (12.9, 18.0)	0	14.1 (12.4, 16.1)	0	12.7 (10.5, 15.5)
0.05	0.3	15.4 (13.0, 18.1)	0.6	14.3 (12.6, 16.3)	1.0	13.1 (10.8, 15.8)
0.2	1.2	15.7 (13.4, 18.5)	2.4	15.1 (13.4, 17.0)	4.0	14.2 (11.9, 17.0)
0.8	4.8	17.4 (14.6, 20.6)	9.6	18.4 (15.6, 21.6)	16.0	19.8 (15.4, 25.4)
Females						
0	0	17.2 (14.6, 20.2)	0	22.8 (20.1, 26.0)	0	33.3 (27.6, 40.2)
0.05	0.3	17.3 (14.8, 20.3)	0.6	23.2 (20.5, 26.3)	1.0	34.2 (28.6, 41.0)
0.2	1.2	17.8 (15.2, 20.8)	2.4	24.4 (21.7, 27.4)	4.0	37.1 (31.5, 43.8)
0.8	4.8	19.6 (16.6, 23.2)	9.6	29.7 (25.3, 34.9)	16.0	51.7 (40.6, 65.8)

[a]The percentage of the initial particle available for long-term clearance is the intercept of the clearance function fit to the data in the Random Effects model (95% confidence intervals).

APPENDIX A

PROTOCOL AMENDMENT 9 TO

"TWO-YEAR REPEATED INHALATION EXPOSURE OF F344 RATS TO HM"

Inhalation Toxicology Research Institute
Protocol Amendment Approval[a]

Protocol No. FY 90-010
Amendment No. 9

Title of Protocol: Two Year Repeated Inhalation Exposure of F344 Rats to HM

ITRI Project Cost Code No.: 5640 **Type of Protocol:** [ ] Regular [X] GLP

Purpose of Protocol Amendment: Check appropriate selections

[ ] Issuance of Animals _____ (species)
[X] Experimental Design Change
[ ] Issuance of Chemicals
[ ] Procedural Change
[ ] Other: _____

A. **Investigator Approval:**	**Signature**	**Date**
Janet Benson ITRI Study Director		10-5-94

B. **Scientific Approval:**	**Signature**	**Date**
Janet Benson ITRI Project Coordinator		10-5-94
Charles N. Hobbs ITRI Program Manager		11/18/94
NA ITRI Assistant Director - Boecker, Lechner, or Lopez		
Charles N. Hobbs ITRI Associate Director - Hobbs		11/18/94
ITRI Director - Mauderly		

C. **Sponsor Approval:** [X] Yes [ ] No If Yes, Sponsor Name: W. Mark Lafranconi

W. Mark Lafranconi — 12/14/94
Sponsor Representative Signature — Date

[a]requirements for approval signatures are on page 2 of this approval form.

(Form revised: 9/94)

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Inhalation Toxicology Research Institute
Protocol Amendment Approval

Protocol No. FY90-010
Amendment No. 9

D. **Institutional Requirements Approval:**

Signature	Date
NA -	-
ITRI Health Protection Unit Staff Member - Schleyer or Hall	
NA (AH 11-15-94)	NA (AH 11-15-94)
ITRI Animal Research Committee Member - Burt	
Dorothy Harris	11-15-94
ITRI Quality Assurance Unit Staff Member - Harris	
NA (AH 11-15-94)	NA (AH 11-15-94)
ITRI Office of Research Administration - Lechner	
Human Subject/Materials Derived from Humans	-

E. **Resource Availability Approval:** The Study Director obtains signatures from persons responsible for research services listed below that will play a major role in the proposed research.

	Signature	Date
Analytical Chemistry – Bechtold	-	-
Animal Use: Dog/Primates – Muggenburg	-	-
Animal Use: Rodents – Burt	-	
Electron Microscopy – Hahn	-	-
Exposure Operations – Barr	-	-
Necropsy – Hahn	-	-
Histopathology – Hahn	-	-
Clinical Pathology Laboratory – Hahn	-	-
Radioactive Measurements – Snipes	-	-

REQUIRED PROTOCOL AMENDMENT APPROVAL SIGNATURES

- **Study Director, Project Coordinator, Program Manager, Health Protection Unit Staff Member, Quality Assurance Unit Staff Member:** All protocol amendments require these signatures. If the Study Director is a Program Manager, the signature of the responsible Assistant Director is required.
- **Office of Research Administration:** All protocols that use humans as experimental subjects.
- **Animal Research Committee Member:** All protocol amendments that use animals as experimental subjects.
- **Assistant Director and Director:** All protocol amendments that use human and nonhuman primates; any use of invasive procedures in the dog or sacrifice of dogs; GLP studies; or cost greater than 500 K.
- **Sponsor:** GLP studies and other studies as determined by contractual agreements.
- **Resource Availability:** See list for current responsible persons.

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PROTOCOL AMENDMENT No. 9
TO
ITRI PROTOCOL No. FY90-010
"Two Year Repeated Inhalation Exposure of F344 Rats to HM"

AMENDMENT:

1. Evaluation of Remaining Brain Slides

The study sponsor, Procter & Gamble has requested that the ITRI perform additional work on Study FY90-010. For completeness the remaining brain sections from the mid and low-dose male rats of the basic study group will be evaluated. The same number of tissue sections have been taken from the same locations as were sampled from the high dose and control basic study group rats. The sections have been stained with hematoxylin and eosin. The sections will be evaluated by Dr. Jack Harkema, the original study pathologist. All brain pathology data from the basic study animals will be summarized in a table (similar to Table 19 of the Final Report) in an amendment to the Final Report. Sections of brain from low and mid-dose female rats, stained with hematoxylin and eosin have already been prepared.

male

error not on 40# 1-22-95

2. Additional Statistics on the Particle Clearance Experiments

The sponsor also requests that additional statistical analyses be performed on the particle clearance data. Specifically, clearance half-times from day 40 post exposure will be analyzed. The following procedures will be followed.

a. Prior to evaluating treatment group effects, homogeneity of variances should be tested by Bartlett's test. If Bartlett's test is significant ($p < 0.05$), the data will be

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analyzed by nonparametric analogs of procedures described below. Prior to evaluating time effects, a test for normality will be conducted, such as the Shapiro-Wilk statistic as implemented in the SAS univariate procedure.

b. For each set of particle exposures (6, 12, or 20 mo), the calculated terminal phase $t_{1/2}$ values will be analyzed to determine if dose has a significant effect on clearance. The general test is obtained from an analysis of variance. Comparisons of each treated group to the control group will be made using Dunnett's t-test. A test for trends could be conducted using appropriate contrasts of means.

c. The calculated terminal phase $t_{1/2}$ values within each dose across time, (6, 12, and 20 mo) will be analyzed across time using repeated measures. A test for trends could be conducted using appropriate contrasts of means.

d. Analyses for male and female rats will be conducted separately.

The results will be summarized in a table and the biological significance of the findings will be discussed in the amendment to the Final Report.

APPENDIX B

LISTING OF ANIMALS EVALUATED FOR

BRAIN LESIONS UNDER PROTOCOL AMENDMENT 9

Table B-1

Male Rats Whose Brains Were Evaluated Under Protocol Amendment 9

B100	B151	B171	C203	C237
B101	B152	B177	C206	C242
B116	B156	B180	C213	C244
B127	B157	B181	C216	C246
B131	B158	B183	C220	C252
B132	B161	B185	C225	C256
B133	B164	C189	C229	C271
B134	B166	C196	C230	C282
B143	B167	C198	C234	

Table B-2

Female Rats Whose Brains Were Evaluated Under Protocol Amendment 9

B471	B506	B542	B563	C614	C643
B472	B507	B543	C565	C617	C644
B475	B509	B548	C568	C619	C647
B482	B513	B549	C573	C621	C649
B483	B524	B552	C577	C624	C655
B491	B526	B553	C585	C625	C656
B494	B528	B555	C594	C626	
B495	B530	B556	C595	C636	
B497	B536	B558	C598	C640	
B503	B538	B559	C601	C641	
B504	B541	B561	C602	C642	

APPENDIX C

DESCRIPTION OF THE STATISTICAL MODEL

The general method for the analysis of these data was a random effects model for longitudinal data. This type of method was used because the experiment involved multiple measurements of pulmonary retention in individual animals exposed to tracer particles at three different times after the start of exposure. Retention functions were estimated using linear regression on the logarithm of the measurements at the three times. Because interest was focussed only on the later component of clearance after 40 days, the retention function was a linear function of time with a single intercept and slope as a function of time after exposure to the tracer particle. The variations of the coefficients of these retention functions among animals were modeled as being random coefficients, where the expected values of the coefficients depends upon population covariates, e.g., exposure concentration, gender, and time on study.

The model used in this report were described by Nan M. Laird and James H. Ware (Random effects model for longitudinal data, *Biometrics 38*: 963-974, 1982). Let Y_i be a vector of logarithm of observations of subject *i*

$$Y_i = X\beta + U_i$$

where X is a known design matrix for the population parameters describing the systematic part of the model, β the unknown coefficients for the population parameters, and

$$U_i = Z_i b_i + e_i$$

with $b_i \sim N(0, \Phi)$ being the distribution of the random coefficients, Z_i being a known design matrix describing the individual covariates for subject *i*, and $e_i \sim N(0, \sigma^2 I)$. This type of model was used rather than a more traditional repeated measures model because of the missing data due to the deaths

of some of the animals during the course of the study. These types of models are a generalization of traditional repeated measures to other types of random effects structures.

The design matrix, X, associated with the systematic portion of the model described the dependence of the slope and intercept on the population covariance of gender, exposure concentration and time on study. The design matrix, Z_i, associated with the random coefficient portion of the model described the slope and intercept of the retention function at the three times on study s being the random coefficients. At these times the logarithms of the retention measurements were fit to a linear function of time with Z_i being

$$\begin{pmatrix} 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 43 & 57 & 78 & 99 & 127 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 43 & 57 & 78 & 100 & 128 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 43 & 57 & 78 & 99 & 127 \end{pmatrix}$$

The first two rows are for the slopes and intercepts of the animals after 6 months on study, the next two rows after 12 months on study, and the last two rows after 20 months on study. Thus, three separate bivariate distributions of random coefficients for the slope and intercept were estimated for the three times at which retention was measured.

The population covariates available in this study were time on study, exposure concentrations, and gender. After consideration of the design it was decided to use a product of the exposure concentration and the time on study at which the exposures to the tracer particles were made. This product of concentration and time will be referred to as the CT product and its value will be denoted by c_t. It was used because the animals were chronically exposed and it provides a better estimate of the dose. Time on study was still used as a population covariate because it reflects aging of the animals, and will be referred to as age of the animal because its values cover the major portion of the

rats lifespan. Thus, there were three population covariates of gender, age, and the CT product. The effect of these covariates and their interactions on the slope and intercept of the retention functions were considered.

The estimation of the coefficients of the model, shown in Table C-1, was made using program 5V of the BMDP statistical package. This program computed the coefficients by the EM algorithm using restricted maximum likelihood estimation. The optimal model was chosen based upon Akaike's information criteria (AIC). Also, estimates of coefficients were evaluated in relation to their standard error to determine if they were significantly different from zero, assuming asymptotic normality. In this model this appeared to be equivalent to making judgements based upon the AIC.

Table C-1

Estimated Coefficients

Covariate (units)	Parameter	Coefficient Estimate	Asymptotic Standard Error
Intercept			
Intercept		2.68	0.084
Gender[a]		0.110	0.085
Age (months)	t	0.0172	0.0063
CT Product (months mg HM/m^3)	c_t	0.0275	0.0085
Interaction of Gender[a] and Age (months)	t	–0.0300	0.0058
Slope (days)			
Slope	d	–0.00988	0.00043
Gender[a]	d	–0.00420	0.00043
Standard Error of the Model	σ		0.176

[a]A mean value parameterization was used for gender, so that the value of the coefficient is added for males and subtracted for females.